# EFFECT OF SOLVENT AND EXTRACTION CONDITIONS ON ANTIOXIDATIVE ACTIVITYOF SAGE (*SALVIA OFFICINALIS L.*) EXTRACTS OBTAINED BY MACERATION

**ORIGINAL SCIENTIFIC PAPER**

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**ABSTRACT: In this paper, the extraction of phenolics from sage (Salvia Officinalis L.) which was grown in the plantations of MP Ljekobilje Trebinje, was carried out by maceration at different extraction time periods (30, 60, 90 , 120, 150 and 180 min), using different organic solvents (40%, 50% and 60% ethanol, 40%, 50% and 60% methanol) and water. The influence of each solvent on the extraction of total phenolics, flavonoids and antioxidative activity of obtained extracts was evaluated. The effect of solid-to-solvent ratio (1:7, 1:10 and 1:15) on the extraction yield of total phenolics was investigated. The influence of different extraction temperatures (room temperature, 30, 40, 50 and 60 ºC) on the content of total phenolics and flavonoids and antioxidative activity of the extract was also investigated. The content of total phenolics and flavonoids in the obtained sage extracts was determined spectrophotometrically. The radical scavenging capacity was determined by the DPPH method, wherein the extract concentration required to neutralize 50% of the initial DPPH radical concentration was also determined. The aqueous solutions of ethanol gave the highest yield of the extract, ie. the highest content of total phenolics and flavonoids in extracts obtained at room temperature and optimal extraction time of 60 minutes. Increasing the time of extraction has increased the content of the total phenolics and flavonoids in extracts, while the excessive time and temperature of the extraction had a negative effect on total phenolics, flavonoids and antioxidative activity of sage extracts.**

**KEYWORDS: solvent extraction, sage extract, total phenolics, flavonoids, antioxidative activity.**

## INTRODUCTION

A free radical may be defined as a molecule or molecular fragment containing one or more unpaired electrons in its outermost atomic or molecular orbital [1]. The presence of unpaired electrons makes these species unstable and very reactive to the interaction with other molecules, because they need to pair the electrons and to make up a more stable compound [2]. Free radicals are produced either from normal cell metabolisms in situ, or from external sources (pollution, cigarette smoke, radiation, medication) [3] and are important intermediates in natural processes involving cytotoxicity, control of vascular tone, and neurotransmission [4].

Because radicals have the capacity to react in an indiscriminate manner leading to damage to almost any cellular component, an extensive range of antioxidant defences, both endogenous and exogenous, are present to protect cellular components from free radical induced damage [5]. An antioxidant is any substance that when present at low concentrations compared to those of an oxidizable substrate delays or prevents oxidation of that substrate.[6]. A balance between free radicals and antioxidants is necessary for proper physiological function [7]. The human body naturally produces antioxidants, but the process is not 100 percent effective in case of overwhelming production of free radicals and that effectiveness also declines with age [8]. If free radicals overwhelm the body's ability to regulate them, a condition known as oxidative stress ensues [7]. Oxidative stress carries harmful effects to all the body systems and is implicated in the pathogenesis of various diseases including hypertension, atherosclerosis, diabetes mellitus and cancer [9].

Strategies such as diet and exogenous antioxidant supplementation may have a potential role in combating oxidative stress caused as a result of environmental factors[10]. Among food components fighting against chronic diseases, great attention has been paid to phyto-chemicals, plant derived molecules endowed with steady antioxidant power[11]. There are a number of epidemiological studies that have shown inverse correlation between the levels of established antioxidants/phytonutrients present in tissue/blood samples and occurrence of cardiovascular disease,

cancer or mortality due to these diseases [12]. Sources of natural antioxidants are primarily plant phenolics that may occur in all parts of plants [13]. Plant phenolic compounds are secondary natural metabolites that commonly possess an aromatic ring bearing one or more hydroxyl substituents. These compounds are often reffered to as „polyphenols" [14]. The antioxidant capacity of phenolic compounds is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers or metal chelators [15].

The most common classification of phenolic metabolites distinguishes the flavonoid and non-flavonoid compounds [16]. Among phenolic compounds found in plants, flavonoids are the most widely studied, with respect to their antioxidant and biological activities [17]. Owing to their omnipresence and impressive biological functions/activities they continue to be thoroughly investigated as potential drugs or food supplements [18].

The increasing consumers demands to acquire healthier fruits and vegetables as well as the urgency in looking to natural compounds with antioxidant activity and enhanced antimicrobial activity against antibiotic-resistant pathogenic bacterial strains have encouraged a quick expansion of research studies about enhanced phenolic extraction and identification methods [19]. In spite of the development of new extraction techniques, classic extraction dominates in many laboratories, mainly due to its simplicity and low economic outlay [20]. The existing techniques of classic extraction, like Soxhlet extraction, maceration and hydrodistillation use solvent, often coupled with elevated temperature and/or agitation. The efficiency of the process can be widely regulated by the selection of suitable solvents and application of possibly effective terms and conditions of extraction under special pressure on its duration and applied temperature [20]. Antioxidant power is usually related to the phenolic content, and the recovery of phenolic compounds from plant materials is influenced by the extraction technique, the extraction time and temperature, the solvents used, the solvent to solid ratio, however, many phenolic compounds are subject to degradation or undergo undesirable oxidation [18].

In this paper an experimental study was conducted to investigate the influence of various solvents and extraction conditions of maceration on total phenolics, flavonoids and antioxidative activity of sage extracts.

## EXPERIMENTAL

For the experimental part of the work, plant material was provided - dried leaves of sage (*Salvia officinalis* L.), that has been planted at the plantations of MP Ljekobilje Trebinje. Plant material was grinded in the electric coffee grinder.

In this study were used: ethanol (95-96%), methanol (99.5%), chlorogenic acid (99%), distilled water, catechin hydrate, Folin Ciocalteu reagent, $AlCl_3$, 0.135 mM solution of 2,2-diphenyl-picylhydrazil (DPPH), an aqueous solution of $Na_2CO_3$ (20%), sodium hydroxide and sodium nitrite (5%). Different concentrations of aqueous solutions of ethanol and methanol (40%, 50% and 60%) were prepared by dilution of organic solvents with distilled water.

Determination of the degree of plant material fragmentation was performed using vibratory sieves and using the equation:

$$\frac{100}{d} = \sum \left(\frac{m_i}{d_i}\right)$$

Where: $m_i$ is the mass fraction presentage, $d_i$ is the intermediate ratio of i – fraction and d is the degree of fragmentation.

Extraction of plant material by maceration method was performed using 40%, 50% and 60% aqueous ethanol and methanol, as well as distilled water. For each solvent, a 10 g of grinded plant material was introduced into the Erlenmeyer of 250 ml, and then poured with a solvent, whereby solid-to-solvent ratio was 1:10.

The procedure was carried out at different time intervals (30, 60, 90, 120, 150 and 180 minutes) at room temperature, stirring the mixture occasionally.Upon expiration of the required time of extraction, the content of the Erlenmeyer was filtered through Whatman No. 40 filter paperusing a Buchner funneland the volume of the filtrate was recorded. The liquid extract was supplemented with the same solvent to the initial volume (100 ml) and left in the refrigerator until analysis. The content of total phenolics in obtained extracts was determined in order to evaluate their yield depending on the time of extraction, and estimate the optimum extraction time and solvent. The total phenolic content (TPC) was determined quantitatively using the Folin Ciocalteu reagent [21], with chlorogenic acid as standard and expressed as milligrams of chlorogenic acid equivalents per gram of dry sage (mg CAE/g).

Total flavonoid content (TFC) in extracts obtained by using different solvents at optimum

extraction time was also examined. TFC was measured using the aluminium chloride colorimetric assay, according to Markham [22] and expressed as milligrams of catechin equivalents per gram of dry sage (mg CE/g). The effect of solid-to-solvent ratio on the extraction yield (%) of total phenolics was also investigated.

Further extraction procedure was carried out at optimum time and using the optimum solvent at higher temperatures (30, 40, 50 and 60ºC), to evaluate the effect of extraction temperature on the content of total phenolics and flavonoids, whereby the heating was provided by using water bath.

To evaluate the antioxidant activity of extracts obtained by using different extraction solvents and temperatures, the radical scavening capacity (RSC) for each extract wereassessed by the method described described in [23] and calculated according to the following equation:

$$RSC_{\%} = \frac{A_c - A_s}{A_s} \cdot 100$$

Where: $A_c$ is is the absorbance of control and $A_s$ is the absorbance of sample.

Based on the known RSC value of each extract, the IC50 value (mg/ml), which presents the concentration of extract required to inhibit 50% of the initial free radical (DPPH) concentration, was estimated from the %RSC versus concentration plot, using a non-linear regression algorithm.

## RESULTS AND DISCUSSION

Grinded dry plant material was separated into fractions by particle size, using the vibratory sieves. The calculated degree of fragmentation was 0.206 mm.

For each individual concentration of an aqueous solution of methanol and ethanol (40%, 50% and 60%) as well as distilled water as a solvent, six samples of dry grinded sage were subjected to maceration at different extraction time (30, 60, 90, 120, 150 and 180 min) and room temperature (25.6°C). The obtained values of the extraction yield of total phenolicsfrom *Salvia officinalis* are shown in Figure 1.

As seen in Figure 1, the highest yield of total phenolics in sage extracts was obtained using a 40% aqueous ethanol solution, which confirms that water-ethanol solvents are probably the most suitable for extraction of phenolic compounds from the sage due to the different polarity of the bioactive constituents, and the acceptability of this solvent system for human consumption [24]. Since the highest yield of phenolic extracts by using 40% ethanol (13.711%), 60% ethanol (7.0256%), 40% methanol (6.3196%), 60% methanol (6.734%) and distilled water (4.2199%) was obtained at extraction time of 60 minutes, the aforementioned was selected as the optimum extraction time in the continuation of the study.

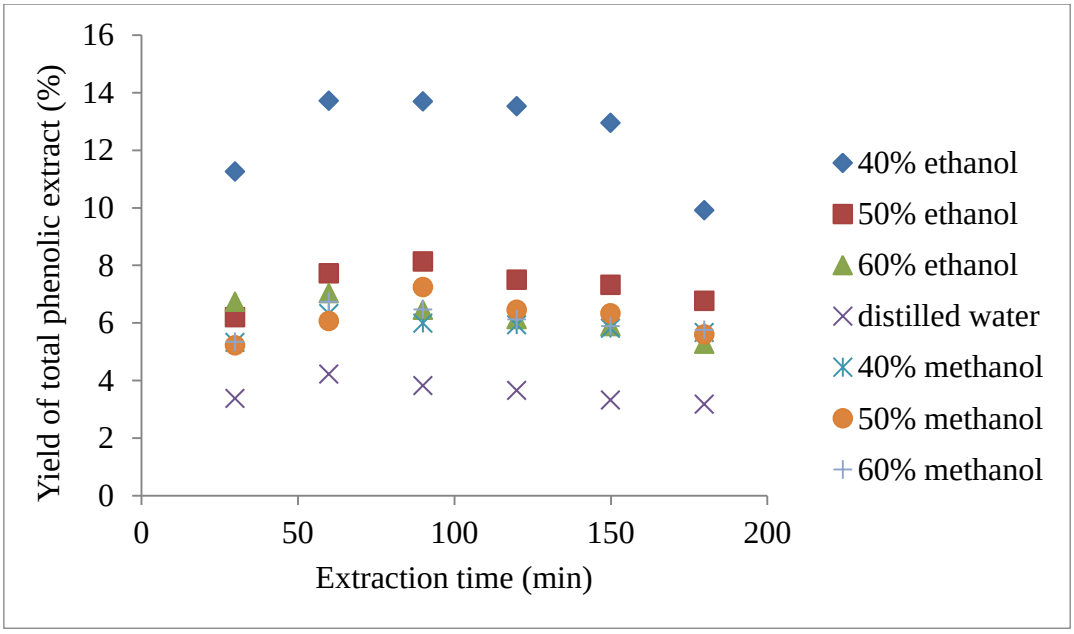


**Figure 1.** Effect of extraction time on the yieldof total phenolics in sage extract

The extraction yield of total phenolics obtained by using 40% and 60% ethanol, 40% and 60% methanol and distilled water was reduced at extraction time above 60 minutes. This phenomenon can be explained as hydrolysis and oxidation of phenolic compounds which may occur due to a longer time of extraction [25]. In case of 50% ethanol and 50% methanol, the yield increased during the first 90 minutes of extraction, indicating differences in the structure of phenolic compounds that also determine their solubility in solvents of different polarity [26], and decreased by extending the extraction beyond that time period. The highest yields of phenolic extract by using 50% ethanol (13.711%) and 50% methanol (7.0256%) were obtained at extraction time of 90 minutes.

To evaluate the effect of solid-to-solvent ratio on the extraction yield of total phenolics, 40%ethanol was selected as solvent. The extraction procedure was carried out at solid-to-solvent ratio of 1:7, 1:10 and 1:15 for 60 minutes at room temperature. The yield of phenolic extract at solid-to-solvent ratio of 1:7 was 7.8952%, while at solid-to-solvent ratio of 1:10 was 13.7110%. The obtained values are in accordance with the laws of mass transfer, according to which the increase of solvent volume affects the increase of the concentration gradient between the plant material and the surrounding liquid phase (solvent), thereby increasing the diffusion of dissolved matter from plant material into liquid mass. However, the lowest phenolic yield was obtained at solid-to-solvent ratio of 1:15 (5.5116%).Sampath [27] obtained similar results in the phenolics extraction study and explained that excessive solvent volume promotes the extraction of undesired compounds from the plant material that may affect the quality of the desired compounds and decrease the yield also.

In further research, the total flavonoid content was determined in the extracts obtained using 40%, 50% and 60% ethanol, 40%, 50% and 60% methanol and distilled water as solvents and at solid-to-solvent ratio of 1:10 during a 60 minute extraction time, and obtained values are compared with the previously obtained values of the total phenolic content (Figure 2).

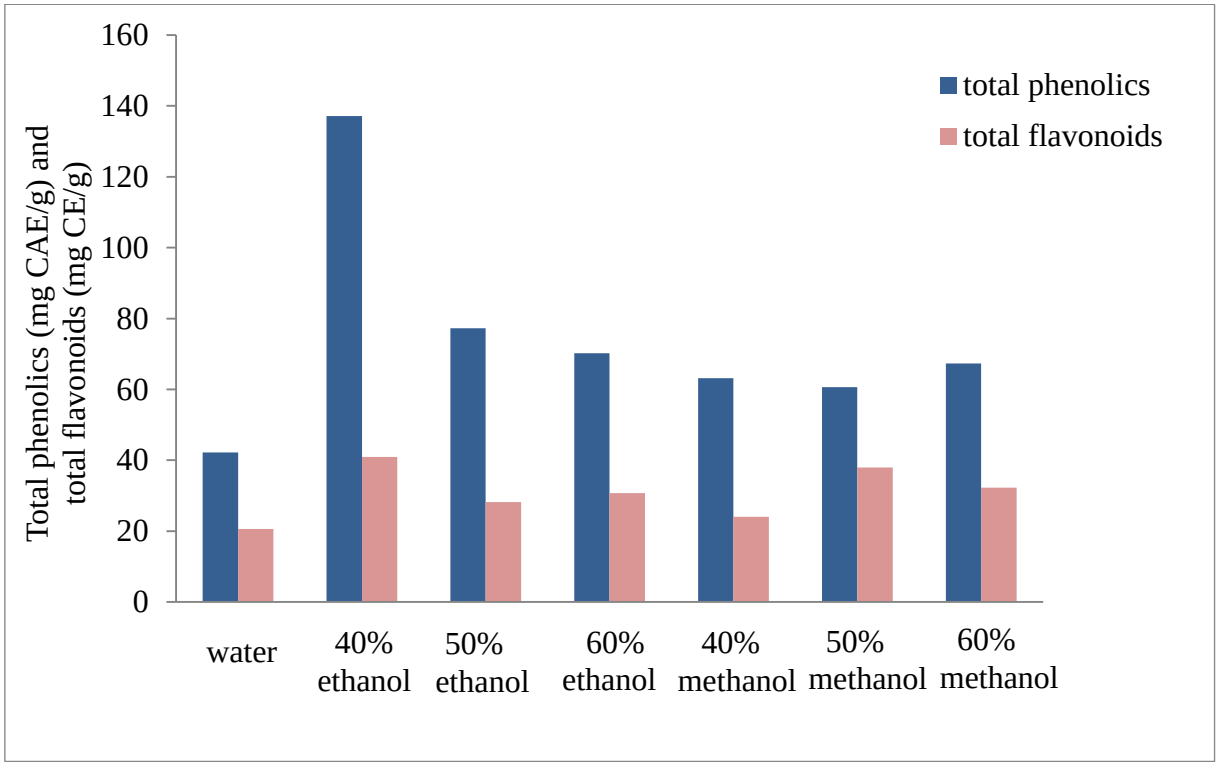


**Figure 2.** Effect of extraction solvent on the content of total phenolics and flavonoids in sage extract

The highest contents of phenolics and flavonoids in sage extracts were obtained by using 40% ethanol as a solvent (137.11 mg CAE/g and 40.912 mg CE/g), and the smallest by application of water as a

solvent (42.199 mg CAE/g and 20.62mg CE/g). The obtained results confirmed ethanol as an optimal solvent for extraction of both total phenolics and flavonoids. Therefore, in further research, the influence of the temperature regime on the content of total phenolics and flavonoids in extract obtained by extraction with 40% ethanol as solvent was investigated.

The research of the effects of different temperature regimes (25.6, 30, 40, 50 and 60°C) was performed by extraction procedure of 60 minutes and solid-to-solvent ratio of 1:10. The obtained results are given in Figure 3.

The results indicate that increasing the extraction temperature reduced the content of total phenolics and flavonoids in sage extracts.

Gradually increasing the extraction temperature from 25.6°C to 60°C the content of total phenolics was reduced from 137.11 mg CAE/g to 65.728 mg CAE/g. Total flavonoid content was reduced from 40.912 mg CE/g to 22.452 mg CE/g. This can be explained by the temperature degradation of phenolic compounds [24].

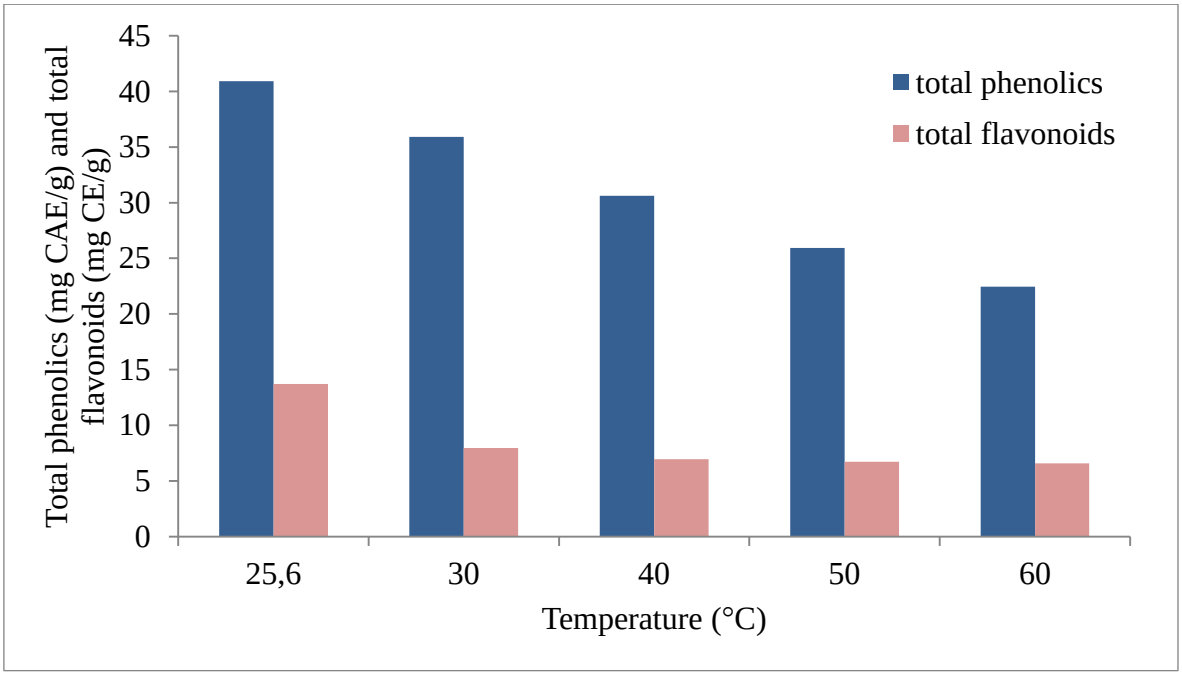


**Figure 3.** Effect of extraction temperature on the content of total phenolics and flavonoids in sage extract

The research of the effects of different solvents on the antioxidative activity of extracts was conducted onextracts obtained by maceration at room temperature during 60 minutes and solid-to-solvent ratio of 1:10.

The obtained concentrations of extracts needed to inhibit 50% of the initial DPPH radical concentration (IC50) are given in Figure 4.

The results show that the extract obtained by 40% ethanol as solvent has the highest antioxidant activity (0.073 mg/ml), while the extract obtained by distilled water exhibit the lowest antioxidant activity (0.355 mg/ml). The observed difference ofantioxidant activitiesis incorelation to the difference of total phenolic contents in mentioned extracts. By increasing the concentration of aqueous ethanol solution ie. by using 50 and 60% aqueous solution of ethanol, extracts of a lower antioxidant activity were obtained (0.075 and 0.078 mg/ml). The extracts obtained by using methanol as solvent showed a fairly uniform antioxidant activity, with the lowest antioxidant activity (0.101 mg/ml) shown by the extract obtained using 40% methanol.

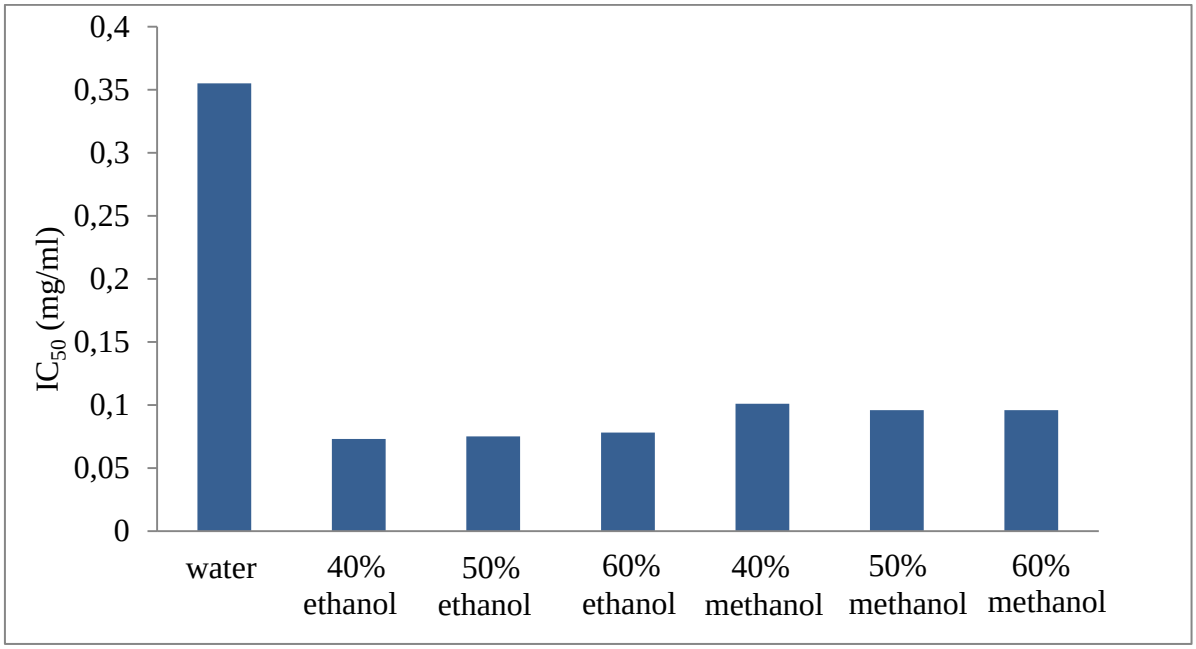


**Figure 4.** Effect of extraction solvent on antioxidant activity of sage extract

The effect of extraction temperature on the antioxidant activity of the extract obtained using 40% ethanol as a solvent was investigated at extraction time of 60 minutes and solid-to-solvent ratio of 1:10. Figure 5 shows IC50 values of extracts obtained at different extraction temperatures. The lowest value of IC50 was determined for the extract obtained at room temperature (0.073 mg/ml), meaning that the extract exhibited the highest antioxidant activity, i.e. the highest ability of free DPPH radicals inhibition. Further increase of extraction temperature resulted in an increase in IC50 values of extracts, which varied at different temperature values (0.101 mg/ml at 30°C; 0.084 mg/ml at 40°C; 0.096 mg/ml at 50°C and 0.088 at 60°C).

Heating might soften the plant tissue and weaken the phenol–protein and phenol–polysaccharide interactions, therefore more polyphenols would migrate into the solvent [28], but some unwanted components can also be extracted, which may reduce the antioxidant activity of the extract.

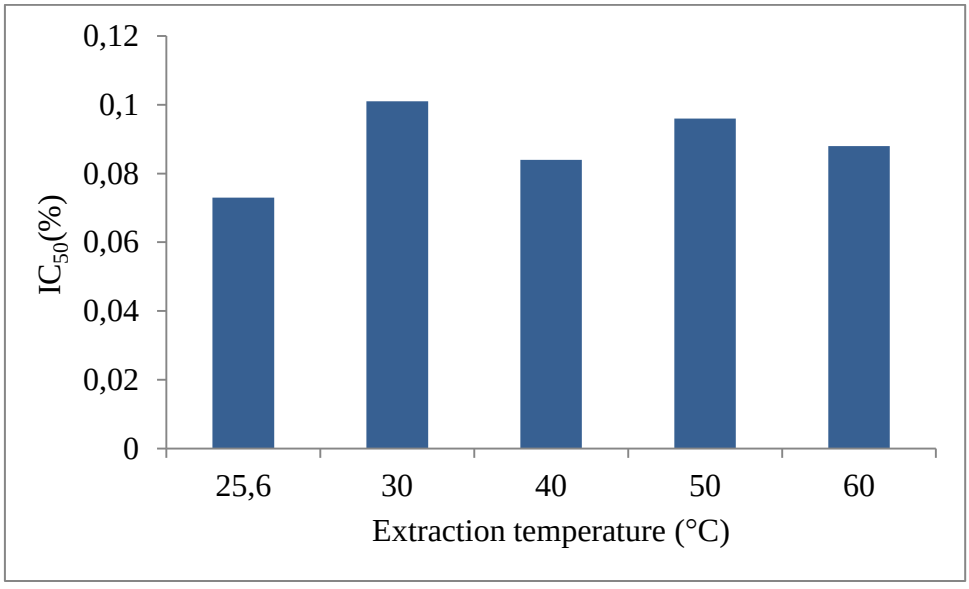


**Figure 5.** Effect of extraction temperature on antioxidant activity of sage extract

## CONCLUSION

The results of the present research on extraction of antioxidants from Salvia officinalis by maceration showed that type of solvent, solid-to-solvent ratio, extraction time and temperature influence the content of total phenolics, flavonoids and the antioxidative activity of phenolic extracts. By extraction of sage using distilled water, 40%, 50%, 60% aqueous ethanol, 40%, 50% and 60% aqueous methanol at room temperature, the phenolic extract yield increased by extraction time, for all solvents used. Most of the solvents gave the highest phenolic extract yield after 60 minutes of extraction. The deviations showed 50% ethanol and 50% methanol, which achieved maximum yield after 90 minutes.Longer extraction time leads to hydrolysis and oxidation of phenolic compounds, which results in a reduction in the phenolic extract yield. The aqueous solutions of ethanol have been shown to be the most suitable solvents for the extraction of phenolics from sage, wherein the solvents with a higher water content showed better extractive properties. Solid-to-solvent ratio can significantly affect the phenolic extract yield, where the increase in solvent volume increases the extraction of phenolics but can also promote the extraction of compounds that affect the quality and the yield of the extract. Increasing the extraction temperature has a negative effect on the content of total phenolics and flavonoids in extracts due to their thermal degradation.

It has been shown that the content of total phenolics and flavonoids in sage extract contributes to increasing its antioxidant activity. Extract obtained by using 40% ethanol as solvent at room temperature and 60 minutes of extraction time had the highest value of IC50, due to the highest content of phenolic compounds.

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